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Comparison of Prevalence of Anti-Hepatitis C Virus Antibodies in Differing South American Populations

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Very little is known about the distribution of hepatitis C virus (HCV) within South America. To assess the exposure of the general population to this virus, a number of sera obtained from three distinct geographical and racial groups were screened using a combination of immunoassays. Initial screening was undertaken with an in-house immunoassay (core-ELISA) using synthetic peptides based on the N-terminus of the HCV core protein. Sera which were repeatedly positive by core-ELISA were also assessed using a commercial third-generation assay. The highest prevalence rate (2.3%) was seen in sera taken from the Tumaco region of Colombia. Lower rates were found in sera taken from La T, Ecuador (0.7%) and Las Majadas, Venezuela (0.7%). This indicates significantly different prevalence in different racial and geographical groups within the region.

KEY WORDS: seroprevalence, HCV, Latin America

INTRODUCTION

Hepatitis C virus (HCV) has been identified as the major cause of posttransfusion hepatitis [Choo et al., 1989, 1990]. Infection leads to chronic liver disease in more than 50% of infected individuals and is a public health problem worldwide [Alter, 1989; Alter et al., 1992]. The prevalence varies widely from country to country but appears to be lower in industrialised nations than in developing countries [Esteban et al., 1989; Tibbs et al., 1991; Coursaget et al., 1990; Nkengasong et al., 1995]. Almost nothing is known of the prevalence of HCV infection in Latin America.

To investigate the epidemiology of HCV in different communities in this area, a strategy based on a combination of immunoassays was used. It has been shown that different commercial immunoassays can differ widely in their sensitivity and specificity and calls have been made for their improvement [Feucht et al., 1995]. A strategy that combines data from different tests is the most cost-effective approach for screening large numbers of sera [Callahan et al., 1993; Schreeberger et al., 1994].

An immunodominant epitope is located at the N-terminus of the core protein [Sällberg et al., 1992; Nasoff et al., 1991] and immunoassays based on this region are highly specific and sensitive as diagnostic tests [Kotwal et al., 1992; Claeys et al., 1992; Sällberg et al., 1992; Nakagiri and Ichihara, 1995]. An in-house immunoassay (core-ELISA) based on a combination of peptides spanning this region was used to screen a large number of sera from different areas and racial groups in Latin America. Sera consistently positive by this assay were also assessed using a commercial third-generation assay.

MATERIALS AND METHODS

Study Population

One thousand two hundred and fifty-three sera samples taken from three populations were used in the study. The first samples studied were taken from the Tumaco region of Colombia, located on the southern Pacific coast on the border with Ecuador. The population is of predominantly West African descent (92%), but also contains *mestizos* (descendants of European and Amerindian peoples) and Amerindians [5% and 3%, respectively; Amador et al., 1992]. Principals activities include forestry, cultivation of plantain, cacao, and fruit and subsistence fishing. A total of 430 sera were used from this region.

The second group of 537 sera samples was taken from La T, a small isolated urban community located in northern Ecuador between the rivers Canandé, Castillo, and Pambil. Income is mainly from production of plantain, cacao, coffee, and livestock. The population in this area is predominantly *mestizo* but also contains a small proportion of people of West African descent [Sempértegui et al., 1993].

The third group of 282 sera samples was obtained from the municipality of Las Majadas, northeastern Bolivar State, South Venezuela. The population consists of peo-

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ple of *mestizo* and Amerindian descent [Noya et al., 1994].

Sera samples were obtained from blood taken by venipuncture following the guidelines laid down by The Ministries of Health of the respective countries.

Control Sera

Forty-eight sera samples containing anti-hepatitis C antibodies (positive controls) were obtained from the Blood Transfusion Service of the Colombian Red Cross (Cruz Roja, Santafé de Bogotá, Colombia). These samples were collected from blood donors over a 3-year period. All were shown to be strongly positive for anti-hepatitis C antibodies using a commercial kit (UBI HCV EIA 4.0, Organon, Teknika, Boxtel, The Netherlands). The presence of anti-hepatitis C antibodies was confirmed in our laboratory using two commercial kits: Abbott HCV EIA 3.0 (Abbott Laboratories, Santafé de Bogotá, Colombia) and HCV Bioscreen (Heber Biotec S.A., Havana, Cuba). Thirty-nine additional positive controls, identified by serology and genotyped by polymerase chain reaction (PCR), were used in the estimation of sensitivity of the core-ELISA. Their identification and genotyping have been reported previously [Watson et al., 1996]. Fifty sera samples were obtained from healthy donors (negative controls) and were shown to be negative for hepatitis C antibodies using two commercial tests (Abbott and HCV Bioscreen).

Sera identified as positive for hepatitis A virus (HAV), human immunodeficiency virus (HIV), herpes simplex virus (HSV) II, rubella, and cytomegalovirus (CMV) infection using commercial serology kits were obtained from the Blood Transfusion Service of the Colombian Red Cross. Samples were identified using the following kits: HAV (HAV BEIA, Abbott Laboratories), HIV (Organon, Teknika), HSV II (Incstar), Rubella IgG (Enzygnost, Merck), and CMV (Enzygnost, Merck).

Study Design

All test sera were screened initially using an in-house peptide-based core-ELISA. This assay is based on a mixture of two synthetic peptides derived from the conserved N-terminal region of the core protein of HCV. Sera that showed reactivity to core peptides were tested using a commercial third-generation ELISA assay (Abbott HCV EIA 3.0). Tests were carried out according to manufacturer's instructions.

Peptide Synthesis

Two peptides, with the sequences ^{1}MSTNPKPQIKTKRNTNRRPQ^{20} and ^{21}DVKFPGGGQIVGGVYLLPRR^{40} [Ching et al., 1992], were synthesised by the solid phase multiple peptide synthesis technique [Merrifield, 1986; Houghten, 1985]. MBHA resin (0.7 meq/g), t-Boc amino acids, and low-high cleavages were used in the process. Once synthesised, peptides were extracted with 10% acetic acid and water, and purity estimated by high-performance liquid chromatography (HPLC). Peptide molecular weights were confirmed by mass spectrometry using a ProteinTOF mass spectrometer (Bruker Analytical Systems, Inc., Billerica, MA, USA).

Core-ELISA

Briefly, Nunc-Immuno MaxiSorp F16 modules (Nunc, Roskilde, Denmark) were coated with 1 μg of antigen mixture per well in 0.05 M sodium carbonate coating buffer, pH 9.6, and incubated overnight at 4°C. After washing with phosphate-buffered saline/Tween 0.5% (PBS/T), plates were blocked with 2% bovine serum albumin (BSA) for 1 hr at room temperature. Plates were washed with PBS/T and used immediately or stored for up to 7 days at 4°C. Sera samples were diluted 1:20 in dilution buffer (PBS/T with 2% low-fat milk powder/1% BSA/2% foetal calf serum) to give a final volume of 100 μL and incubated for 1 hr at room temperature. After washing three times with PBS/T, 100 μL of dilution buffer containing a 1:1,000 dilution of anti-human IgG conjugate (Sigma, St. Louis, MO) was added to each well and incubated for 1 hr at room temperature. Plates were washed five times with PBS/T and 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate (Kirkegaard & Perry Laboratories, MD, USA) was added to each well. After incubation at room temperature for exactly 5 min, the reaction was stopped by the addition of 100 μL of 1 M phosphoric acid (Sigma). Optical densities were measured at 450 nm on a Labsystems Multiskan MS plate reader (Labsystems, Helsinki, Finland). Positive and negative controls were included in each plate.

Statistical Analysis

Data were processed using Epi-Info 6 software (Centers for Disease Control and Prevention, Atlanta, GA).

RESULTS

Performance of the Core-ELISA

Cut-off point. The cut-off point was determined from the measurements of 50 controls taken from healthy donors and 48 sera of patients confirmed as seropositive for HCV by three commercial tests. The cut-off point was chosen arbitrarily as the formula (average of negative controls × 3) + (average of positive controls × 0.15)/2. This value appeared reasonable as it allowed the accurate differentiation of the positive and negative control sera. Three positive and three negative control sera were included in each plate and interassay variation was calculated to be ≤10.2%.

Sensitivity. The core-ELISA identified correctly 38 of 39 (97%) of PCR-positive sera tested and none of 50 (0%) negative control sera by a blind assay, suggesting that the assay was highly sensitive.

Specificity. In a control group of sera, identified by serology with antibodies to hepatitis A (n = 9), HIV (n = 3), HSV II (n = 9), rubella (n = 10), and CMV (n = 10), no reactivity was found to the core-ELISA.

Results of Screening

The results (Tables I and II) show that the overall prevalence of hepatitis C antibodies in the study groups was 1.2%, but that it varied depending on the geographi-

TABLE I. Prevalence of Anti-HCV Antibody in Different Populations Studied

	Tumaco, Colombia	La T, Ecuador	Las Majadas, Venezuela
Total number of sera	430	545	264
Sera positive in core-ELISA	30	4	2
% sera positive in core-ELISA	6.9%	0.7%	0.7%
Sera confirmed positive with commercial kit	10	4	2
% of confirmed positives	2.3%	0.7%	0.7%

TABLE II. Prevalence of Anti-HCV Antibody by Sex and Age Group

	Males		Females		Total prevalence	
Age group (years)	No. positive/no. tested	%	No. positive/no. tested	%	No. positive/no. tested	%
1–14	0/272	0.0	4/229	1.7	4/501	0.8
15–30	0/177	0.0	0/153	0.0	0/330	0.0
31–45	5/120	4.2	2/116	1.7	7/236	3.0
46–60	1/65	1.5	1/49	2.0	2/114	1.7
>60	2/40	5.0	1/18	5.5	3/58	5.2
All	8/674	1.2	8/565	1.4	16/1239	1.2

cal and racial group studied. The highest prevalence (2.3%) was seen in sera taken from the Tumaco region of Colombia. Lower rates were seen in sera taken from La T, Ecuador (0.7%) and Las Majadas, Venezuela (0.7%). A good correlation was seen between the results of the in-house core-ELISA and the commercial test for sera from Ecuador and Venezuela–4/4 (100%) and 2/2 (100%), respectively. However, of 30 sera samples taken from the Tumaco region and shown to be reactive for anti-core antibodies, only 10 (33%) were confirmed as containing HCV antibodies by the commercial test.

Table II shows the prevalence of anti-HCV antibodies correlated to sex and age groups for all sera tested. The incidence of HCV infection is equal in both males and females (1.2% and 1.4%, respectively) and increases with age (from 0.8% in the 1–14 year age group to 5.2% in the >60 age group). The relationship between prevalence and age was shown to be linear using chi square for linear trend of Epi-Info 6 (value = 9.33, $P = 0.0022$).

DISCUSSION

Commercial kits for the detection antibodies against hepatitis C virus and other viral diseases are often prohibitively expensive for large-scale screening in developing countries. We have therefore used an in-house kit based on peptides together with a confirmatory commercial assay to study seroprevalence of HCV in several regions of South America.

The prevalence of anti-HCV antibodies in the La T region of Ecuador and the Las Majadas region of Venezuela appears relatively low (0.7% in both cases). These figures are similar to those calculated for industrialised nations, where prevalence has been estimated at between 0.5% and 1.5% of the adult population [Kuo et al., 1989; Esteban et al., 1989; Roggendorf et al., 1989].

However, a higher prevalence (2.3%) of anti-HCV antibodies was observed in sera samples from the Tumaco region of Colombia. This suggests a higher exposure to HCV that could be due to lifestyle factors, geographical differences, or environmental factors. The Tumaco and La T populations also contain a higher proportion of people of West African descent. Given the high rates of HCV infection found in certain parts of Africa [Coursaget et al., 1990; Nkengasong et al., 1995], it is possible that there exists a historical association of HCV with this population. We are currently serotyping samples from these regions using a peptide-based system to learn more about the distribution and origin of subtypes in the region.

Other studies in Peru [Hyams et al., 1992] and Venezuela [Muller et al., 1992] have shown seroprevalence of HCV being mostly confined to high-risk populations—such as haemophiliacs and haemodialysis patients—with very low seropositivity in low-risk populations (as low as 0% for sera samples taken from the northern jungle region of Peru where the sampled population is described as mainly of *mestizo* and Amerindian descent). No evidence of HCV infection was found in a recent survey of an isolated community of Yukpa Indians in western Venezuela [Blitz-Dorfman et al., 1995].

The difference between the number of sera samples identified as reactive in the core-ELISA—but not in the commercial assay—was notable in the Tumaco region. Several possible explanations exist. Previously unidentified subtypes could be circulating in the region, which are detected by the core-ELISA, but not by the commercial kits. Kits using recombinant proteins derived from NS4 (5-1-1 and c100-3) have been shown to be poor antigens for nontype 1 genotypes [Chan et al., 1991; McOmish et al., 1993; Maggi et al., 1995; Zein et al., 1995]. It is also possible that high levels of antibodies with nonspecific binding activity affected the core-ELISA to a greater extent than the commercial kit causing these aberrant results. All of the areas in this study are located in malarious zones and polyclonal, nonspe-

cific activation of B cells has been shown to be a consequence of malaria infection [Banic et al., 1991].

Unfortunately, data on disease incidence are not available for these study regions and therefore the prevalence of anti-HCV antibodies cannot be correlated with liver disease. However, it seems likely that these data, demonstrating the existence of HCV antibodies in these areas of South America, will be important in the diagnosis of liver disease by health workers in the region. The correlation between increasing age and increasing exposure to HCV seen in the whole study group is as expected and has been shown previously [Frommel et al., 1993]. This correlation was shown to be linear and significant ($P < 0.05$).

In summary, it appears that the prevalence of anti-HCV antibodies in the predominantly *mestizo* populated regions of South America included in this study is similar to that shown for industrialised nations and that the likelihood of infection increases linearly with age. The incidence of infection is higher in the racially distinct Tumaco region and studies of these populations are in progress.

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